

THE PERIOD OF QUIESCENCE IN THE RESPONSE TO LIGHT
BY AMOEBA
(THE RESPONSE TO LIGHT BY AMOEBA)

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INTRODUCTION

As is well known, an amoeba, when subjected to sudden illumination, responds by cessation of movement, and, as this cessation does not take place immediately, an interval exists between the increase in illumination and the reaction, thus giving rise to a reaction-time. As shown in a previous paper (Folger, 1925), the duration of the reaction-time depends on various factors, such as intensity of the light, previous stimulation, physiological condition of the animal. However, the reaction-time does not constitute the whole of the response to light by amoeba. After stopping, the animal remains motionless for some time, then resumes locomotion. The interval during which movement is suspended and which has been designated the quiescent period or period of quiescence is likewise dependent upon numerous factors and must necessarily be included in a complete investigation of the response to light by the organism.

Investigations dealing with a phase in the reaction of light similar to the one just mentioned have not been numerous. Possibly the growth reaction in a mold, as described by Blaauw (1914), may be compared to it. Blaauw was able by means of mirrors to illuminate the spore-carrier of *Phycomyces nitens* on four sides simultaneously, thus bringing about a symmetrical stimulation and avoiding bending. He found that after an illumination of one minute an increase in the rate of growth could be detected about three and a half minutes later, though this reaction-time was subject to much variation, depending both on the individual and on the intensity of the light. The increase in growth was augmented more and more until in some cases it amounted to as much as two hundred or even four hundred per cent of the normal rate. Shortly afterward, however, growth began gradually to slow down until finally it fell below normal, a condition which was of short duration for soon the plant was growing at its usual rate again.

Apparently more closely related to the phase to be considered in *Amoeba* is that encountered in streaming plant cells such as those of *Chara* and *Nitella*, as described by Ewart (1903), who gives an excellent review of previous work on protoplasmic streaming and adds the results of experiments by himself. Unfortunately for work with illumination, the cells of both of these plants contain chloroplasts, and photosynthesis brings in a complicating factor which interferes somewhat with a study of other effects of light. But in the case of several additional stimulating agents, especially mechanical shock, there is a response similar to that given by *Amoeba* to sudden illumination. The response of a streaming cell of *Chara* to mechanical shock, for instance, comprises a cessation followed shortly by a resumption of movement,

and, as Ewart shows, a close relationship exists between the intensity of the stimulating agent and the time during which streaming remains suspended.

Some use has already been made of the period of quiescence in an investigation of the reactions of *Amoeba*. Folger (1926) demonstrated that in response to mechanical shock it becomes longer with increase in the magnitude of stimulation. This paper is concerned with a study of the quiescent period in the response of *Amoeba* to light.

MATERIALS AND METHODS

Apparatus similar to that used in the experiments to be presented has already been described (Folger, 1925). Light was obtained from a 1000-watt, 112-volt, cylindrical Mazda stereoptican lamp and flashed upon the amoebae by means of the plane mirror of the microscope. Heat rays were eliminated by placing a vessel of distilled water between lamp and mirror. The voltage of the current was about 114, and as the amoebae were placed at a distance of 25 centimeters from the light, they were, consequently, subjected to an illumination of approximately 16,000 meter candles intensity, even after some absorption of light by mirror and water. Active specimens of *Amoeba proteus* were used as experimental animals.

EXPERIMENTAL RESULTS

Mention has been made of the lengthening of the period of quiescence with increase in the magnitude of mechanical shock. In the experiments referred to, the shock was brought about by dropping pieces of wire through a glass tube to the slide on which the amoebae had been placed. Under these conditions the time of application of the stimulus did not vary, it might be described as instantaneous, and the distance through which the weights fell was not changed. Consequently the intensity of the shocks varied with the weights of the pieces of wire, which were all of the same diameter but of various lengths. In the experiments now to be described, the intensity of the stimulating agent, that is, the light, remained unchanged, but it was easy enough to vary the magnitude of the stimulus by altering the time of exposure. The following effects of light on the period of quiescence were investigated: (1) the effect of changing the length of exposure; (2) the effect of altering the time elapsing between exposures; (3) the effect of repeated exposures.

Relation between the period of quiescence and the exposure period

If one keeps the intensity of the light constant but alters the time during which it acts, it is immediately apparent that the quiescent period varies with the time of exposure. This is well illustrated by the results of the experiment recorded in Table 1, in which an amoeba was subjected to light for periods ranging from 4 seconds to 45 seconds. A response was obtained in every instance, with an average reaction-time of about 2.6 seconds.¹ As can be seen, it was quite otherwise with the period of quiescence. An exposure of 4 seconds produced a quiescent period of 10.6 seconds; one of 10 seconds, a quiescent period of 18.7 seconds; and one of 15

¹ It is not to be assumed that length of exposure can have no effect on the reaction-time, but to produce a reaction-time essentially different from that given above would require a much shorter exposure than any used in this experiment.

seconds, a quiescent period of 24 seconds. Thus the period of quiescence increased with increase of exposure and this continued until the exposure amounted to 45 seconds, when the period of quiescence was 42 seconds.

Further analysis brings out other relationships between exposure and quiescent periods. In the final column is shown the difference obtained by subtracting the former from the latter. With an exposure of 4 seconds the difference amounted to 6.6 seconds. With an exposure of 10 seconds it was 8.7 seconds, and with an exposure of 15 seconds it was 9 seconds. Thus far the rate of increase has been greater in the period of quiescence than in the exposure period, but this did not continue indefinitely, for with an exposure of 20 seconds the difference had fallen to

TABLE I

Showing increase of the period of quiescence with increase of exposure period. Time indicated by seconds.

Exposure period (s)	Number of tests	Period of quiescence (t)			t-s
		Maximum	Minimum	Average	
4	5	14	7	10.6	6.6
10	3	20	17	18.8	8.7
15	3	30	21	24.0	9.0
20	3	32	24	27.0	7.0
25	2	29	27	28.0	3.0
30	2	32	32	32.0	2.0
45	1			42.0	-3.0

7 seconds, with an exposure of 30 seconds it was 3 seconds, and with an exposure of 35 seconds it was 2 seconds. The exposure period is now increasing faster than the period of quiescence, and the ultimate result is shown at an exposure of 45 seconds, when the difference amounted to - 3 seconds. In other words, the exposure period has become longer than the period of quiescence. It has already been stated that the amoeba ceased to move after it was in illumination for about 2.6 seconds. So, when exposed for 45 seconds, it stopped in 2.6 seconds and resumed movement in another 42 seconds, 0.4 of a second before the light was turned off. Obviously an exposure greater than 45 seconds would not have called forth a longer period of quiescence, since the amoeba would still have stopped and then resumed movement within about 44.6 seconds after the light was turned on.

Time required for recovery from the effects of sudden illumination

Reaction by *Amoeba* to light or to mechanical shock is, as previously stated, followed by a refractory period, which may be either absolute or relative; that is, the amoeba may refuse to react to a second stimulus, or it may react but with an altered response. Partial recovery from the effects of either kind of stimulus is indicated by a reaction-time that is longer than that obtained after complete recovery (Folger, 1925, 1926). The effect of partial recovery from sudden illumination on the period of quiescence is illustrated by the results of the experiment shown in Table II.

TABLE II

Showing the influence upon the period of quiescence on the lack of recovery from the effects of previous stimulation. Light turned on and left on until amoeba resumed locomotion.

Time in dark previous to exposure	Number of tests	Period of quiescence (sec.)		
		Maximum	Minimum	Average
4 seconds	4	No reaction		
10 seconds	4	30	18	22.5
30 seconds	6	40	15	24.8
1 minute	6	60	25	36.7
2 minutes	6	75	40	54.2
10 minutes	1			175.0
30 minutes	1			220.0
2 hours	1			218.0

In this experiment a single amoeba was used and was subjected to a series of tests in each of which it was first exposed to light for 1 minute, then to darkness for 3 minutes, and again to light, this time for 30 seconds. This procedure was intended to bring about a condition of uniformity. Following the 30-second exposures the light was turned off for intervals varying in the several trials from 4 seconds to 2 hours. Finally in each case the amoeba was then exposed to illumination and left exposed until the characteristic response was completed by the resumption of flow. As shown in the table, no reactions were obtained when 4 seconds were allowed for recovery. Ten seconds permitted a recovery sufficient to obtain reactions in every instance, with an average period of quiescence in 4 trials of 22.5 seconds. Thirty seconds for recovery resulted in an average period of quiescence of 24.5 seconds; 1 minute for recovery, in an average period of quiescence of 36.7 seconds; and 2 minutes for recovery, in an average period of quiescence of 54.2 seconds. Ten minutes, 30 minutes, and 2 hours for recovery were followed by periods of quiescence of 175 seconds, 220 seconds, and 218 seconds, respectively. Clearly the period of quiescence became longer as the amoeba recovered from the effects of previous stimulation, reaching a maximum when recovery was complete. Evidently under the conditions of the experiment complete recovery was not attained until after 10 minutes but before 30 minutes had elapsed. This contrasts with the 1 or 2 minutes necessary to obtain a minimum reaction-time (Folger, 1925, Alsup, 1937).

A few further words of explanation are necessary for a proper evaluation of the results just given. When an amoeba is exposed to light and ceases to move, the stoppage is generally sudden, permitting accurate timing with a stopwatch. If the exposure period is not too long or if too much time has not elapsed between stimuli, the resumption of movement also occurs with sufficient abruptness to be timed with a fair degree of precision. However, when a strong light is combined with sufficient time for complete or almost complete recovery from the effects of previous stimulation and with a long exposure period, especially with one so long that the amoeba begins to move while the light is still acting, the resumption of flow cannot be ascertained with the same accuracy. Under these conditions the first indication of movement is usually an extrusion of a number of small pseudopodia, followed by an erratic flow, first in one direction, then in another. Gradually these

pseudopodia lessen in number, movement becomes stronger, until finally the amoeba is again flowing briskly. If, now, one considers the first indication of movement as the end of the period of quiescence, it is easily determined. But if he assumes that this period is not completed until the amoeba has resumed the more vigorous movement that obtained before stimulation, he must exert a judgement, and the period can not be timed with the same degree of accuracy. In the present instance the period of quiescence was interpreted as ending when the protoplasmic flow became as strong as it was before stimulation, but even if the first indication of movement had been chosen as the final point in the reaction, while the higher figures in the preceding table would have been altered by several seconds, in the very long exposures even by as much as 25 or 30 seconds, the general aspect of a curve drawn from them would not be materially changed.

The effect of repeated stimulation

Repeated stimulation by light, with a short time intervening between exposures, brings out another peculiarity in the behavior of amoeba, which is exemplified by the results of the experiment summarized in Table III. This table records averages

TABLE III

Showing decrease in period of quiescence with repeated exposures. Each amoeba subjected to darkness for at least 10 minutes and then given 10 successive 3-second exposures to light, with intervals of 1 minute in darkness between exposures. Number of animals in cultures 1, 2, 3, were 11, 13, and 16, respectively.

Reaction number	Average period of quiescence (sec.)			General average
	Culture No. 1	Culture No. 2	Culture No. 3	
1	18.5	21.6	14.3	18.1
2	17.8	18.6	14.4	16.9
3	12.6	14.0	14.6	13.7
4	11.6	14.3	12.6	12.8
5	10.4	12.4	12.4	11.7
6	8.8	11.9	10.1	10.3
7	10.8	11.5	11.9	11.4
8	10.4	9.4	10.8	10.2
9	8.8	8.2	9.9	9.0
10	10.3	10.1	8.8	9.7

of the reactions of from 11 to 16 amoebae from each of 3 cultures. Each amoeba was subjected to darkness for at least 10 minutes and then given 10 successive 3-second exposures to light, with intervals of 1 minute in darkness between exposures. Though there were variations among the animals from the same culture and even more among animals from diverse cultures, the general behavior was the same in every case and is reflected in the total averages given in the table. The average period of quiescence for the first exposure amounted to 18.1 seconds, for the second exposure to 16.9 seconds, for the third exposure to 13.7 seconds. As one may see, this decline continued until the sixth exposure, when the period of quiescence was 10.3 seconds. In the 4 succeeding trials at no time did it fall far below 10 seconds

and on the tenth exposure amounted to 9.7 seconds. Even when further tests were added, as they were in some instances though the results are not given in the table, there was no further reduction in the period of quiescence.

This diminution of the period of quiescence with repeated exposure is somewhat reminiscent of the phenomenon known as *treppe*, to be met with in the physiology of muscle.

DISCUSSION

The response to light that has been under consideration is intimately related to locomotion and must perhaps in final analysis be explained in terms of amoeboid movement. According to Mast (1926), an amoeba consists essentially of an inner fluid plasmasol and an outer solid plasmagel, which is surrounded by a very thin elastic surface membrane, the plasmalemma. The plasmasol is hypertonic and the plasmagel and plasmalemma act as semipermeable membranes, resulting in the development of an osmotic pressure in the plasmasol and a stretching of the plasmagel, especially at these points where it is weakest, where the pseudopodia are formed. Locomotion, according to Mast, involves a continuous change from plasmasol to plasmagel at the anterior end of the animal and from plasmagel to plasmasol at the posterior end. For further analysis of amoeboid movement one is referred to Mast's paper. For our purpose it will suffice to point out that when it is moving, the amoeba must be in a state of dynamic equilibrium. Illumination might cause an increase in the elastic strength of the plasmagel, especially at the tip of the advancing pseudopodium (Mast, 1932), bringing about a temporary breakdown in the established equilibrium, with consequent cessation of forward movement. Shortly an equilibrium is reformed and the animal again moves. Since experiments described in the preceding section have shown that stimulation applied shortly after the resumption of locomotion either fails to bring about a reaction or is followed by a reaction with a shortened period of quiescence, it is apparent that the new equilibrium is by no means at the same level as that which was in existence before the cessation occurred. Otherwise one would expect identical responses when the one stimulation is followed by another of equal strength. The experiments show, moreover, that the original level is gradually restored since a stimulus of a given intensity will finally elicit a second response of exactly the same magnitude as that which it called forth at first. Obviously, the time during which the disturbed equilibrium is reverting to the original level constitutes the refractory period.

SUMMARY

1. *Amoeba proteus* reacts to sudden illumination by cessation of movement. The time during which it is motionless has been designated the period of quiescence.

2. The period of quiescence becomes longer with extension of the time of exposure to illumination. At first the period of quiescence increases more rapidly than the exposure period, but soon the rate of increase of the latter becomes the greater, with the final result that the amoeba begins to move while the light is still on.

3. Stimulation by light is followed by a refractory period, which may be absolute

or relative; that is, the amoeba may either refuse to respond to a second stimulus, or it may react but with an altered response. Incomplete recovery from the effects of previous stimulation results in a period of quiescence that is shorter than that obtained after complete recovery.

4. If an amoeba is repeatedly exposed to illumination at intervals of a minute, the period of quiescence is at first relatively long, but becomes progressively shorter, arriving at a minimum after about 6 minutes.

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